

THAT WHICH IS CLAIMED:

1. A method of preparing a chemically modified hemoglobin solution comprising:

5 (a) contacting a stroma free hemoglobin solution with at least one filtration means, wherein a first filtration means retains viral particles and allows passage of a filtrate comprising hemoglobin and endogenous antioxidant enzymes and the filtrate is substantially free of viral contamination;

(b) chemically modifying the filtrate with an agent; and,

10 (c) isolating a composition comprising a chemically modified hemoglobin and antioxidant enzyme, wherein at least one endogenous antioxidant polypeptide retains enzymatic activity.

2. The method of claim 1, wherein at least one of the endogenous antioxidant enzymes retaining enzymatic activity is selected from the group consisting of superoxide  
15 dismutase, catalase, and glutathione peroxidase.

3. The method of claim 1, wherein said first filtration means allows the passage of at least 50% of the endogenous antioxidant enzymes.  
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4. The method of claim 1, wherein the filtration means comprises an AG Technology 500,000 molecular weight cutoff filter.

5. The method of claim 1, wherein said first filtration means reduces the  
25 passage of viral particles that are between about 200-25 nm in size.

6. The method of claim 5, wherein said first filtration means reduces the passage of viral particles that are 80-100 nm in size.

30 7. The method of claim 5, wherein said first filtration means reduces the passage of viral particles that are between about 80-50 nm in size.

8. The method of claim 5, where said first filtration means reduces the passage of viral particles that are between about 50-25 nm in size.

5 9. The method of claim 1, wherein said first filtration means reduces the passage of said viral particles by about 3 to about 10 log units.

10. The method of claim 1, wherein said first filtration means produces a filtrate having a viral load reduction of at least 3 log units.

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11. The method of claim 1 further comprising contacting the filtrate with at least one second filtration means wherein said second filtration means allows the passage of hemoglobin and endogenous antioxidant enzymes and retains virus particles.

15 12. The method of claim 11, wherein the second filtration means comprises a filter selected from the group consisting of Pall DV-50 filter, Pall DV-20 filter, and Millipore Viresolve NFR.

20 13. The method of claim 1, wherein the modifying agent is a bifunctional modifying agent.

25 14. The method of claim 13, wherein said modifying agent is selected from the group consisting of sebacyl chloride, glutaraldehyde, diasprin derivatives, polyaldehydes, polyoxyethylene, dextrans, and inulin.

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15. The method of claim 13, wherein the modifying agent is bifunctional polyoxyethylene.

30 16. The method of claim 1, wherein the modifying agent is a mixture of bifunctional and monofunctional polyoxyethylene.

17. The method of claim 15, wherein the modified hemoglobin solution is PHP.

5 18. The method of claim 1, wherein the chemical modification further comprises deoxygenation and pyridoxalation of the hemoglobin.

19. The method of claim 1, wherein the viral contamination of said isolated modified hemoglobin solution comprises a viral titer of less than about 1 TCID<sub>50</sub> unit/ml.

10 20. The method of claim 1, wherein the chemically modified hemoglobin solution comprises about a 50% to about a 200% increase in endogenous red blood cell antioxidant activity per unit of hemoglobin found in red blood cells.

15 21. A method of preparing a chemically modified hemoglobin consisting of:  
(a) contacting a stroma free hemoglobin solution with at least one filtration means, wherein a first filtration means retains viral particles and allows passage of a filtrate comprising hemoglobin and endogenous antioxidant enzymes and the filtrate is substantially free of viral contamination;  
20 (b) chemically modifying the filtrate with an agent; and,  
(c) isolating a composition comprising modified hemoglobin and endogenous antioxidant enzymes.

22. A hemoglobin solution comprising a chemically modified hemoglobin and  
25 at least one endogenous antioxidant enzyme, wherein said modification comprises attachment of a POE linkage, said endogenous antioxidants retain enzymatic activity, and said solution is substantially free of viral contamination.

23. The modified hemoglobin solution of claim 22, wherein said modified  
30 hemoglobin is PHP.

24. The modified hemoglobin solution of claim 22, wherein the viral contamination of said solution comprises a viral titer of less than about 1 TCID<sub>50</sub> unit/ml.

5 25. The modified hemoglobin solution of claim 24, wherein the viral titer of said particles that are 25-30 nm in size is less than about 1 TCID<sub>50</sub> unit/ml.

26. The modified hemoglobin solution of claim 25, wherein said viral particle is hepatitis A.

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27. The modified hemoglobin solution of claim 24, wherein the viral titer of viral particles less than 70 nm in size is less than about 1 TCID<sub>50</sub> unit/ml.

28. The modified hemoglobin solution of claim 27, wherein the viral particle is hepatitis A or hepatitis C.

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29. The modified hemoglobin solution of claim 22, wherein said endogenous antioxidant enzyme is selected from the group consisting of superoxide dismutase, catalase, hemoglobin peroxidase, and glutathione peroxidase.

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30. The modified hemoglobin solution of claim 22, wherein said solution contains between a 50% to 200% increase in antioxidant activity per unit of hemoglobin found in red blood cells.

25 31. The modified hemoglobin solution of claim 22, wherein said solution comprises the chemically modified hemoglobin and at least superoxidide dismutase, catalase and at least one additional endogenous antioxidant enzyme.

30 32. A method of decreasing the level of nitric oxide present in the circulation of a mammal, said method comprising, administering to a mammal in a need thereof a

therapeutically effective amount of the modified hemoglobin solution of claim 22 in a pharmaceutically acceptable carrier.

33. The method of claim 32, wherein said modified hemoglobin is  
5 administered to a mammal having systemic hypotension.

34. The method of claim 32, wherein said modified hemoglobin is administered to a mammal having septic shock.

10 35. A method of treating red blood cell loss, said treatment comprising administering to a mammal in need thereof a therapeutically effective amount of the modified hemoglobin of claim 22.